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Synthesis and preliminary biological screening of 6-aminopyrazolo[3,4-b]pyridine derivatives

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ABSTRACT

*6-chloro-3-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**1**) was utilized as key intermediate for the synthesis of new 6-amino derivatives (**2-17**) by heating with a number of aliphatic amines. Heating **1** with aromatic amines under similar conditions failed to give the corresponding amino derivatives. The new compounds were fully characterized and some of them were preliminary screened for anticancer, COX inhibition and antimicrobial activities. The compounds are not cytotoxic and some of them are potent and selective COX-2 inhibitors. In particular compound 6-benzylamino-3-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**4**) with IC_{50} = 0.11 µM and SI = 33 for COX-2 . 6-Hexylamino-3-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (**6**) exhibited antifungual and antibacterial activities (Gram -ve) comparable to the reference drugs. The results show clearly that the natur of N-substituent significanlty affect the biological activity.*

Keywords: 6-aminopyrazolo[3,4-*b*]pyridines, Nucleophilic substitution, cytotoxicity, COX inhibition, antimicrobial

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INTRODUCTION

Pyrazolo[3,4-b]pyridines are attractive condensed heterocyclic compounds and are reported to possess diverse range of biological and pharmaceutical activities such as antitumor [1], antibacterial [2], anti-inflammatory [3], inhibitors of protein kinase [4], cyclin-dependent kinase 1 (CDK1) [5], glycogen synthase kinase-3 (GSK-3) [6], and HIV reverse transcriptase [7]. In the other hand, the amination of heteroaryl halides is of central importance in the synthesis of pharmaceutically relevant molecules [8]. Small molecules containing nitrile and amino groups which are easily converted into other functional groups are important components of many pharmaceuticals [9]. However, the amination of compounds containing pyridinyl halides with nitrile group seems to be not easy. Due to that the amination condition usually needs a stronger base and a higher temperature the nitrile group might be easily react under such conditions [10]. Hence, the synthesis of nitrile substituted aminopyridines has potential challenges, we were then interested in the synthesis and evaluation of biological properties of 6-aminopyrazolo[3,4-c]pyridine derivatives.

MATERIALS AND METHODS

Chemistry

Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, SMP3, Staffordshire, UK) and were uncorrected. Pre-coated silica gel plates (Kieselgel 0.25 mm, 60G F254, Merck, Darmstadt, Germany) were used for TLC monitoring. Visualization of the spots was effected using an ultraviolet lamp (Spectroline, model CM-10, Seattle, USA) (λ= 254 nm). IR spectra were carried out as KBr discs on a

Shimadzu IR-470 Spectrometer (Shimadzu, Kyoto, Japan) at Faculty of Pharmacy, Assiut University. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker 400 MHz spectrometer (Faculty of Pharmacy, Ain Shams University, Cairo, Egypt) or on a Varian EM-360L NMR spectrometer (60 MHz, Varian, CA, USA) at Faculty of Pharmacy, Assiut University, Assiut, Egypt. Chemical shifts are expressed in δ -values (ppm) relative to tetramethylsilane (TMS) as an internal standard using DMSO-d6 as a solvent and deuterium oxide was used for the detection of exchangeable protons. Elemental microanalyses were performed on a Vario elemental analyzer III (Vario, Hanau, Germany) at the unit of Microanalysis, Faculty of Science, Cairo University and at the Regional Center for Mycology and Biotechnology, Microanalytical Unit, Al-Azhar University, Cairo, Egypt.

General method for the synthesis of the target compounds (2-17)

A mixture of 6-chloro-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile **1** [11] (0.268 g, 1 mmol) and the appropriate amine (4 mmol) were heated to 80 ^{o}C overnight with constant stirring. The reaction mixture was cooled to room temperature and taken up in ethyl acetate. The organic layer was washed with 5% aq. $NaHCO_3$, followed by washing with water and then with brine. The organic layer was dried over anhydrous $MgSO_4$ and solvent was removed under reduced pressure. The crude product was recrystallized from aqueous ethanol to give the title compound as white needle-shaped crystal (60-80 % yield):

3-Methyl-1-phenyl-6-(piperidiny-1-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-5- carbonitrile (2)

Yield 80%, Mp 153.5-155 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.83 (s, lH, pyrdin-H), 8.17 (d, *J* = 8 Hz, 2H), 7.52 (t, *J* = 8 Hz, 2H), 7.29 (t, *J* = 8 Hz, H), 3.62 (m, 4H), 2.52 (s,3H, CH_3), 1.62-1.55 (m, 6H, CH). ^{13}C (100 MHz, DMSO-d6) δ (ppm): 162.0, 150, 144.6, 142.1, 140.8, 131.2, 129.6, 128.9, 126.0, 121.9, 120.1, 111.0, 92.0, 50.3, 25.6, 25.0, 24.3, 12.8. Anal. found C, 72.17; H, 6.12, N, 22.19 (%). Calc. for ($C_{19}H_{19}N_5$): C, 71.90; H, 6.03; N, 22.07 (%).

3-Methyl-1-6-(morpholin-4-yl)-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5- carbonitrile (3)

Yield 75%, Mp 198-199 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.97 (s, lH, pyrdin-H), 8.13 (d, *J* = 8 Hz, 2H), 7.52 (t, *J* = 8 Hz, 2H), 7.29 (t, *J* = 8 Hz, 1H), 3.78 (t, *J* = 4 Hz, 4H), 3.62 (t, *J* = 4 Hz, 4H), 2.50 (s,3H, CH_3). ^{13}C (100 MHz, DMSO-d6) δ (ppm): 168.3, 160.5, 149.9, 146.9, 144.7, 142.3, 141.0, 139.4, 139.0, 136.2, 129.6, 128.3, 126.1, 122.3, 120.3, 115.6, 111.1, 91.8, 71.8, 49.5, 12.6. Anal. found C, 67.94; H, 5.44, N, 22.17 (%). Calc. for ($C_{18}H_{17}N_5O$): C, 67.70; H, 5.37, N, 21.93 (%).

6-Benzylamino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (4)

Yield 70%, Mp 204-205.5 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.61 (s, lH, pyrdin-H), 8.23 (t, *J* = 4 Hz, NH), 7.96 (br. d, 2H), 7.43-7.18 (m, 8H), 4.60 (t, *J* = 4 Hz, 4H), 2.50 (s,3H, CH_3). ^{13}C (100 MHz, DMSO-d6) δ (ppm): 157.2, 152.7, 151.0, 148.0, 144.7, 144.5, 140.1, 139.3, 139.2, 139.0, 134.6, 129.3, 128.8, 128.6, 128.5, 127.4, 127.2, 127.0, 125.9, 125.6, 124.0, 120.0, 119.7, 117.6, 112.0, 109.1, 103.3, 88.3, 45.312.5. Anal. found C, 74.50; H, 5.11, N, 20.88 (%). Calc. for ($C_{21}H_{17}N_5$): C, 74.32; H, 5.05, N, 20.63 (%).

6-Cyclohexylamino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (5)

Yield 73%, Mp 214-215 ^{o}C, ^{1}H NMR (60 MHz, $CDCl_3$) δ (ppm): 8.30 (d, *J* = 8 Hz, 2H), 7.98 (s, lH, pyrdin-H), 7.70-7.10 (m, 3H), 5.25 (d, *J* = 8 Hz, NH), 4.40-3.65 (m, 1H), 2.52 (s,3H, CH_3), 2.42-0.7 (m, 10H). Anal. found C, 72.65; H, 6.51, N, 21.37 (%). Calc. for ($C_{20}H_{21}N_5$): 72.48; H, 6.39, N, 21.13 (%).

6-Hexylamino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (6)

Yield 78%, Mp 159-161 ^{o}C, ^{1}H NMR (60 MHz, $CDCl_3$) δ (ppm): 8.25 (d, *J* = 8 Hz, 2H), 7.92 (s, lH, pyrdin-H), 7.80-7.00 (m, 3H), 5.25 (br. t, NH), 3.80-3.30 (m, 2H), 2.50 (s,3H, CH_3), 1.80-0.5 (m, 11H). Anal. found C, 72.31; H, 6.99, N, 21.23 (%). Calc. for ($C_{20}H_{23}N_5$): C, 72.04; H, 6.95, N, 21.00 (%).

6-Dimethylamino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (7)

This compound was obtained as a side product during the reaction of aromatic amines with compound 1 in presence of sodium carbonate or catalytic amount of HCl. Yield 85%, Mp 208.5-210 ^{o}C, ^{1}H NMR (60 MHz, $CDCl_3$) δ (ppm): 8.20 (br. d, 2H), 8.05 (s, lH, pyrdin-H), 7.65-7.05 (m, 3H), 3.50 (s, 6H), 2.60 (s,3H, CH_3). Anal. found C, 69.57; H, 5.51, N, 25.49 (%). Calc. for ($C_{16}H_{15}N_5$): C, 69.29; H, 5.45, N, 25.25 (%).

3-Methyl-1-phenyl-6-(1-phenylethyl)amino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (8)
Yield 68%, Mp 204-206 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.55 (s, lH, pyrdin-H), 7.92 (d, *J* = 8 Hz, 2H), 7.79 (d, *J* = 4 Hz, NH), 7.48-7.42 (m, 4H), 7.32 (d, *J* = 8 Hz, 2H), 7.24 (d, *J* = 8 Hz, 2H), 7.17 (d, *J* = 8 Hz, 2H), 5.15 (m, 1H), 2.41 (s,3H, CH_3), 1.57 (d, *J* = 4 Hz, 3H),. ^{13}C (100 MHz, DMSO-d6) δ (ppm): 156.5, 152.8, 150.8, 150.1, 145.7, 145.4, 144.6, 144.5, 139.3, 139.2, 139.1, 134.1, 129.3, 128.8, 128.6, 128.5, 126.9, 126.7, 126.4, 125.8, 125.6, 122.9, 119.9, 117.8, 109.1, 88.5, 52.0, 33.6, 12.5. Anal. found C, 75.02; H, 5.46, N, 20.06 (%). Calc. for ($C_{22}H_{19}N_5$): C, 74.77; H, 5.42, N, 19.82 (%).

3-Methyl-1-phenyl-6-(2-phenylethyl)amino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (9)
Yield 73%, Mp 172-173 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.66 (s, lH, pyrdin-H), 8.20 (d, *J* = 8 Hz, 2H), 7.79 (t, *J* = 4 Hz, NH), 7.47 (d, *J* = 8 Hz, 2H), 7.33-7.19 (m, 6H), 3.96-3.58 (m, 2H), 2.91 (t, *J* = 8 Hz, 2H), 2.50 (s,3H, CH_3). ^{13}C (100 MHz, DMSO-d6) δ (ppm): 157.4, 157.2, 151.3, 148.6, 144.7, 144.5, 141.5, 138 139.8, 139.6, 139.4, 139.2, 138.9, 133.3, 129.3, 127.0, 126.6, 126.6, 125.6.1, 128.88, 128.6, 128.5, 126.8, 125.8, 88.5, 52.0, 33.6, 12.5. Anal. found C, 75.02; H, 5.46, N, 20.06 (%). Calc. for ($C_{22}H_{19}N_5$): C, 74.77; H, 5.42, N, 19.82 (%).

3-Methyl-1-phenyl-6-(4-phenyl)pierazin-1-yl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (10)
Yield 75%, Mp 159-161 ^{o}C, ^{1}H NMR (60 MHz, $CDCl_3$) δ (ppm): 8.30 (br. d, 2H), 7.95 (s, lH, pyrdin-H), 7.80-6.6 (m, 8H), 3.80 (br. t, 2H), 3.40 (br. t, 2H), 2.55 (s,3H, CH_3). Anal. found C, 73.18; H, 5.74, N, 21.59 (%). Calc. for ($C_{24}H_{22}N_6$): C, 73.07, H, 5.62, N, 21.30 (%).

6-(Butylamino)-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (11)
Yield 80%, Mp 155-156 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.61 (s, lH, pyrdin-H), 8.24 (t, *J* = 8 Hz, 2H), 7.51 (t, *J* = 8 Hz, NH), 7.50-7.28 (m, 2H), 7.27 (t, *J* = 8 Hz, 1H), 3.43-3.35 (m, 2H), 2.45 (s,3H, CH_3), 1.62 (m, 2H), 1.37 (m, 2H), 0.94 (t, *J* = 8 Hz, 3H). Anal. found C, 71.03, H, 6.37, N, 23.21 (%). Calc. for ($C_{18}H_{19}N_5O$: C, 70.80, H, 6.27, N, 22.93 (%).

3-Methyl-1-phenyl-6-(propylamino)-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (12)
Yield 69%, Mp 154-156 ^{o}C., ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.63 (s, lH, pyrdin-H), 8.27 (t, *J* = 8 Hz, 2H), 7.60 (t, *J* = 8 Hz, NH), 7.54-7.50 (m, 2H), 7.27 (t, *J* = 8 Hz, 1H), 3.43-3.35 (m, 2H), 2.45 (s,3H, CH_3), 1.67 (m, 2H), 0.96 (t, *J* = 4 Hz, 3H). Anal. found C, 70.24, H, 5.94, N, 24.37 (%). Calc. for ($C_{17}H_{17}N_5$): C, 70.08, H, 5.88, N, 24.04 (%).

3-Methyl-6-(2-methylpropylamino)- 1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5- carbonitrile (13)
Yield 78%, Mp 188-189 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.50 (s, lH, pyrdin-H), 8.21 (t, *J* = 8 Hz, 2H), 7.48 (t, *J* = 8 Hz, NH), 7.25 (t, *J* = 8 Hz, 1H), 3.33-3.25 (m, 2H), 2.50 (s,3H, CH_3), 2.10 (m, 1H), 0.92 (d, *J* = 4 Hz, 6H). ^{13}C (100 MHz, DMSO-d6) δ (ppm):157.6, 151.5, 151.3, 146.1, 144.7, 142.9, 139.5, 139.0, 138.9, 129.9, 129.3, 127.9, 125.6, 120.7, 119.7, 119.4, 117.7, 115.0, 108.8, 88.1, 49.4, 27.5, 20.7, 12.5. Anal. found C, 71.06, H, 6.34, N, 23.16 (%). Calc. for ($C_{18}H_{19}N_5$): C, 70.80, H, 6.27, N, 22.93 (%).

3-Methyl-1-phenyl-6-(4-methyl)pierazin-1-yl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (14)
Yield 78%, Mp 149-150 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.75 (s, lH, pyrdin-H), 8.16 (d, *J* = 8 Hz, 2H), 7.52 (t, *J* = 8 Hz, 1H), 7.29 (t, *J* = 8 Hz, 1H), 3.46 (t, 4H), 2.60-2.42 (m,7H), 2.23 (s, 3H). ^{13}C (100 MHz, DMSO-d6) δ (ppm):186.2, 160.5, 140.9, 129.6, 127.3, 91.6, 54.6, 49.0, 46.1, 12.60. Anal. found C, 68.91, H, 6.13, N, 25.49 (%). Calc. for ($C_{19}H_{20}N_6$): C, 68.65, H, 6.06, N, 25.28 (%).

6-(Isopropylamino)- 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (15)
Yield 77%, Mp 147.5-148.5 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.50 (s, lH, pyrdin-H), 8.19 (t, *J* = 8 Hz, 2H), 7.49 (t, *J* = 8 Hz, 2H), 7.25 (t, *J* = 8 Hz, NH), 7.01 (t, *J* = 8 Hz, 1H), 4.30 (m, 1H), 2.50 (s,3H, CH_3), 1.27 (d, *J* = 8 Hz, 6H). ^{13}C (100 MHz, DMSO-d6) δ (ppm):156.8, 147.8, 144.7, 144.5, 139.4, 139.1, 130.9, 129.4, 127.6, 125.6, 124.2, 119.9, 117.7, 112.7, 108.9, 88.4, 43.5, 22.0, 12.5. Anal. found C, 70.43, H, 5.96, N, 24.32 (%). Calc. for ($C_{17}H_{17}N_5$): C, 70.08, H, 5.88, N, 24.04 (%).

6-(Allylamino)- 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (16)
Yield 75%, Mp 180-182 ^{o}C, ^{1}H NMR (400 MHz, DMSO-d6) δ (ppm): 8.52 (s, lH, pyrdin-H), 8.17 (t, *J* = 8 Hz, 2H), 7.67 (t, *J* = 4 Hz, NH), 7.47 (t, *J* = 8 Hz, 2H), 7.24 (t, *J* = 8 Hz, 2H), 5.92 (m, 1H), 5.22 (d, *J* = 16 Hz, 1H), 5.22 (d, *J* = 16 Hz, 1H), 5.10 (d, *J* = 16 Hz, 1H), 4.03 (t, *J* = 8 Hz, 2H), 2.26 (s,3H, CH_3). ^{13}C (100 MHz, DMSO-d6) δ

(ppm):157.3, 154.4, 151.1, 145.2, 144.7, 144.3, 139.3, 139.2, 138.9, 136.7, 135.7, 129.4, 129.0, 125.6, 125.4, 119.9, 118.5, 117.6, 117.0, 116.0, 113.8, 109.0, 88.3, 44.2, 12.5. Anal. found C, 70.82, H, 5.21, N, 24.49 (%). Calc. for ($C_{17}H_{15}N_5$): C, 70.57, H, 5.23, N, 24.2 (%).

6-(2-Hydroxyethyl)amino)-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (17)
Yield 80%, Mp 179-180 ^oC, ^1H NMR (400 MHz, DMSO-d6) δ (ppm): 9.19 (br. s, NH), 8.51 (s, lH, pyrdin-H), 8.26 (t, *J* = 8 Hz, 2H), 7.49 (t, *J* = 8 Hz, 2H), 7.23 (t, *J* = 8 Hz, 1H), 3.66-3.40 (m, 4H), 2.48 (s,3H, CH_3). ^{13}C (100 MHz, DMSO-d6) δ (ppm):170.51, 158.2, 151.5, 144.7, 140.1, 132.7, 129.5, 125.6, 119.4, 107.7, 107.4, 59.8, 43.0, 12.6. Anal. found C, 65.92, H, 5.45, N, 24.30 (%). Calc. for ($C_{16}H_{15}N_5O$): C, 65.52, H, 5.15, N, 23.88 (%).

Anticancer activity screening
The cytotoxicity of the synthesized compounds was assayed using the standardized assay procedure of the National Cancer Institute (NCI Bethesda, Maryland, USA).[12]

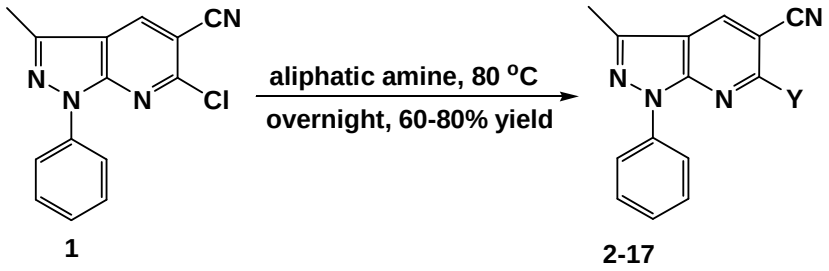
In vitro cyclooxygenase (COX) inhibition assay
The ability of ten of the synthesized compounds (**2-5**, **8-10** and **15-17**) to inhibit both COX-1 and COX-2 isozymes was measured using colorimetric COX (ovine) inhibition using enzyme immunoassay (EIA) kit (Kit catalog number 760111, Cayman Chemical, Ann Arbor, MI, USA) following the manufacturer's instructions and as reported [12-13]. Two standard drugs; indomethacin, one of the oldest and most commonly used NSAIDs, and celecoxib, the prototype selective COX-2 inhibitor, were used as reference compounds (positive control). Different concentrations of reference drugs or tested compounds were incubated with the enzymes for a period of 5 min at 25 ^oC. After the incubation period an addition of the colorimetric substrate and arachidonic acid was done then the absorbance was measured at 590 nm using plate reader. The results are shown in Table 1.

Antimicrobial activity
The Tests were performed according to NCCLS recommendations (National Committee for clinical laboratory Standards ,1993). Screening tests regarding the inhibition zone were carried out by the well diffusion method [14]. The inoculum suspension was prepared from colonies grown overnight on an agar plate, and inoculated into Mueller-Hinton broth (fungi using malt broth). A sterile swab was immersed in the suspension and used to inoculate Mueller-Hinton agar plates (fungi using malt agar plates). The compounds were dissolved in dimethyl sulfoxide (DMSO) with different concentrations (10, 5, 2.5 mg/ml). The inhibition zone was measured around each well after 24 h 37 ˚C (for bacteria) and 72 h (for fungi). Antimicrobial activity was expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated. The data was expressed as mean ± SD. Results are depicted in Table 2.

RESULTS AND DISCUSSION

Chemistry
The target compounds (**2-17**; Table 2) were prepared according to the synthetic pathway outlined in Scheme 1. The synthesis of the new pyrazolopyridine derivatives proceeded through the general intermediate, 6-chloro-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**1**) [11] which was synthesized in a good yield starting from 5-amino-3-methyl-l-phenylpyrazole-4-carboxaldehyde as reported by one of the authors [11]. The synthesis was accomplished by heating compound **1** with the appropriate aliphatic amine (scheme 1). Several attempts to use aromatic amines were unsuccessful. For example, with fusion, heating in DMF under reflux in presence of triethylamine or sodium carbonate [15] or heating in 2-ethoxyethanol under reflux using catalytic hydrochloric acid [16]. This can be attributed to the lower nucleophilicity of aromatic amines. Surprisingly, 6-*N*,*N*-dimethylamino derivative (**7**) was obtained during our attempt to react compound **1** with aromatic amines in DMF. By searching the literature, it was found that the use of DMF to replace active halogen atoms by dimethylamino groups is well known [17].

The compounds crystalize from aqueous ethanol giving beautiful needle-shaped crystals. We recently reported the crystal structure of one of the compounds (**12**) [18]. Generally, the structures of the products were characterized by the presence of N-H signal and aliphatic protons in NMR and the characteristic peak for nitrile group at 2115 cm^{-1} in IR.



Scheme 1. The synthesis of the target compounds (2-17)

Biological screening

Anticancer activity

Anticancer activity of the synthesized compounds was studied using high-efficiency biological screening according to the Developmental Therapeutic Program (DTP) of the USA National Institutes of Health, National Cancer Institute (NCI) (Bethesda, Maryland, USA) [19, 20].

All the prepared compounds were submitted to the National Cancer Institute "NCI" (www.dtp.nci.nih.gov); 4 compounds (**3, 8**, **14**, and **16**) were selected for preliminary screening. The selected compounds with NCI codes NSC: D-791340/1, NSC: D-791341/1, NSC: D-91342/1 and NSC: D-791343/1 were tested at a single dose (10 μM) in the full NCI 60-cell panel. The operation of this screening utilizes 60 different human tumor cell lines, representing leukemia, melanoma, and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. The results for each compound were reported as a mean graph of the percent growth of the treated cells compared to that of the untreated control cells. The compounds didn't show significant cytotoxic activity. These results indicate that the compounds are not cytotoxic and deserve further biological screening.

In vitro cyclooxygenase (COX) inhibition assay

The obtained data (Table 1) showed that all the tested compounds were weaker inhibitors of COX-1 isozyme (IC_{50} = 3.7-11.4 μM range) and exhibited stronger COX-2 isozyme inhibitory activities (IC_{50} = 0.11-1.21 μM range). Also, the results showed COX-2 selectivity indexes in the 8.33–33.4 range. In particular, compound **4** is 33.4 fold more selective for COX-2 compared to COX-1.

Table 1. In vitro COX-1 and COX-2 inhibitory activity and COX-2 selectivity index of 2-5, 8-10, 15-17 and three reference drugs

Entry No.	Compd. No.	IC_{50} (μM)[a]		S.I.[b]
		COX-1	COX -2	
1	**2**	6.55	0.78	8.4
2	**3**	5.41	0.59	9
3	**4**	3.67	0.11	33.4
4	**5**	7.41	0.89	8.3
5	**8**	10.33	1.23	8.4
6	**9**	8.97	0.97	9.2
7	**10**	11.32	1.42	7.9
8	**15**	9.74	0.79	12.3
9	**16**	10.41	1.21	8.6
10	**17**	7.63	0.65	11.7
11	Celecoxib	14.8	0.05	296
12	diclofenac sodium	3.9	0.8	4.9
13	Indomethacin	0.039	0.49	0.08

[a] *IC_{50} value is the compound concentration required to produce 50% inhibition of COX-1or COX-2. The value is the mean of two determinations and deviation from the mean is <10%.*

[b] *Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).*

Analysis of side effects of the older conventional non-steroidal anti-inflammatories shows that the lower the COX-1 inhibition, the lower the overall side effect profile. Four compounds (**2**, **3**, **4** and **17**) showed higher potency and better selectivity than the clinically used drug (Diclofenac sodium®).

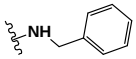
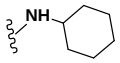
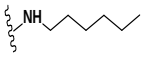
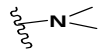
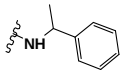
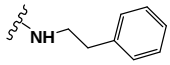
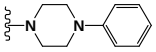
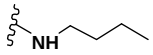
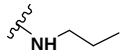
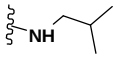
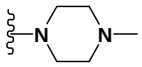
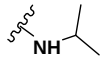
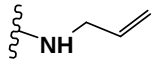
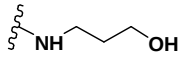
Interestingly, compound **4** exhibited higher selectivity than Indomethacin® but lower than Celecoxib®. It is well-known that balanced COX-1/COX-2 inhibition is required for optimal activity and safety. Celecoxib® and the new

compounds described here are pyrazole derivatives as many other COX-2 inhibitors [21]. The structure activity data acquired showed that the amine should have heterocycle or aromatic moiety for good selectivity.

Antimicrobial

Applying the agar plate diffusion technique, all the synthesized compounds **(1-17)** were screened for their *in vitro* antibacterial activity against Gram positive bacteria and Gram negative bacteria. The antibacterial activity was evaluated against *Staphylococcus aureus (*RCMB 010010), *Bacillus subtilus* (RCMB 010067) and *Escherichia coli* (RCMB 010052), *Pseudomonas aeruginosa* (RCMB 010043). Ampicillin and Gentamycin are used as a standard drug for the comparison of antibacterial activity.

Table 2. The results of antimicrobial screening. Mean zone of inhibition (mm) beyond the diameter produced on a range of pathogenic microorganisms.[a]

microorganisms / Compd. No.	Y	I [b]	II [b]	III [b]	IV [b]	V [b]	VI [b]
1	Cl	15 ± 0.2	25 ± 0.56	20 ± 0.18	14 ± 0.3	15 ± 0.4	NA [c]
2		23 ± 0.38	20 ± 0.27	20 ± 0.18	16 ± 0.1	15 ± 0.2	NA [c]
3		20 ± 0.3	26 ± 0.5	20 ± 0.18	15 ± 0.1	14 ± 0.1	NA [c]
4		22 ± 0.2	19 ± 0.3	20 ± 0.4	12 ± 0.2	15 ± 0.18	NA [c]
5		26 ± 0.7	19 ± 0.27	20 ± 0.28	14 ± 0.17	16 ± 0.1	16 ± 0.15
6		NA [c]	25 ± 0.7	15 ± 0.2	13 ± 0.15	18 ± 0.2	20 ± 0.2
7		24 ± 0.4	24 ± 0.7	16 ± 0.5	14 ± 0.4	17 ± 0.2	20 ± 0.7
8		NA [c]	15 ± 0.2	20 ± 0.3	15 ± 0.2	15 ± 0.18	25 ± 0.7
9		22 ± 0.4	15 ± 0.5	16 ± 0.2	13 ± 0.3	15 ± 0.18	18 ± 0.3
10		20 ± 0.8	14 ± 0.2	16 ± 0.3	13 ± 0.18	14 ± 0.1	NA [c]
11		23 ± 0.28	15 ± 0.2	15 ± 0.2	NA [c]	NA [c]	NA [c]
12		20 ± 0.6	15 ± 0.17	NA [c]	NA [c]	NA [c]	NA [c]
13		20 ± 0.5	20 ± 0.7	NA [c]	14 ± 0.3	NA [c]	NA [c]
14		20 ± 0.2	20 ± 0.6	20 ± 0.28	15 ± 0.2	14 ± 0.18	16
15		16 ± 0.2	23 ± 0.78	NA [c]	14	NA [c]	NA
16		20 ± 0.5	24 ± 0.2	NA [c]	12 ± 0.2	NA [c]	NA [c]
17		20 ± 0.29	23 ± 0.69	20 ± 0.2	15 ± 0.2	14 ± 0.1	NA [c]
Amphotericin B		23 ± 0.1	25 ± 0.1				
Ampicillin				23 ± 0.2	32 ± 0.3		
Gentamycin						17 ± 0.1	19 ± 0.3

[a] Antimicrobial activity was expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated. The data was expressed as mean ± SD.

[b] I: Aspergillus fumigatus (RCMB 02568), II: Candida albicans (RCMB 05036), III: Staphylococcus aureus (RCMB 010010), IV: Bacillus subtilus (RCMB 010067) V: Pseudomonas aeruginosa (RCMB 010043) and VI: Escherichia coli (RCMB 010052).

[c] NA: No activity, RCMB: Regional Centre for Mycology and Biotechnology.

The antifungal activity was evaluated against *Candida albicans* (RCMB 05036) and *Aspergillus fumigatus* (RCMB 02568). Amphotericin B is used as standard drugs for the comparison of antifungal activity. From the antimicrobial testing compounds **1-17,** it is observed that all the newly synthesized compounds show good to moderate level of antibacterial (Gram -ve) and antifungal activity as shown by Table-2.

The data revealed that some compounds (**2-5**, **8** and **9**) have comparable activity to ampicillin® against *Staphylococcus aureus*. However, all compounds showed weak or no activity against *Bacillus subtilus.* On the other hand, compounds (**6** and **7**) showed promising activity against

Escherichia coli and Pseudomonas aeruginosa comparable to Gentamycin®. However, compounds **8** and **9** were more potent against *Escherichia* coli. than against *Pseudomonas aeruginosa*. In general, the compounds showed better antifungal activity than antibacterial. Interestingly compound **5** with *N*-cyclohexyl is the most potent against *Aspergillus fumigatus* with lower activity against Candida albicans. In contrast, compound **6** with *N*-hexyl is the most potent against Candida albicans with much lower activity against *Aspergillus fumigatus.* However, compounds (**2** and **3)** showed similar activity against both fungi that is comparable to Amphotericin B. Some compounds (**11** and **16**) showed good activity against *Aspergillus fumigatus*, while compounds (**7**, **13** and **15**) were more active against *Candida albicans*. The results of antimicrobial screening clearly show that *N*-substituent greatly affect the activity.

CONCLUSION

6-amino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**2-17**) having different amino substituents were synthesized from the corresponding chloro derivative. The compounds were characterized and some of them were submitted to NCI. The results showed that the compounds are not cytotoxic. Therefore, some of them were screened for COX inhibition and antimicrobial activities. One compound (**4**) was found more potent and selective COX-2 inhibitors than Diclofenac sodium® and Indomethacin®. Another compound (**6**) exhibited antibacterial and antifungal activity comparable to the reference drugs, Gentamycin® and Amphotericin B®, respectively. The results show clearly that the natur of substituent at 6- position significanlty affect the biological activity.

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